

# Dispersion compensation in three-photon fluorescence microscopy at 1,700 nm

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**Abstract:** Signal generation in three-photon microscopy is proportional to the inverse-squared of the pulse width. Group velocity dispersion is anomalous for water as well as many glasses near the 1,700 nm excitation window, which makes dispersion compensation using glass prism pairs impractical. We show that the high normal dispersion of a silicon wafer can be conveniently used to compensate the dispersion of a 1,700 nm excitation three-photon microscope. We achieved over a factor of two reduction in pulse width at the sample, which corresponded to over a 4x increase in the generated three-photon signal. This signal increase was demonstrated during *in vivo* experiments near the surface of the mouse brain as well as 900  $\mu\text{m}$  below the surface.

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**OCIS codes:** (260.2030) Dispersion; (190.4180) Multiphoton processes; (170.0180) Microscopy.

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## Introduction

Dispersion management is critical for multiphoton microscopy because ultrashort pulses can be strongly affected by material dispersion [1], which results in reduced signal generation. Dispersion management components are common in two-photon microscopy (2PM) systems [2,3], and 2PM systems have been shown to maintain a laser pulsewidth down to 10 fs [4]. However, glasses commonly used in a microscope generate normal material group velocity dispersion (GVD) at the typical excitation wavelengths for 2PM (600–1300 nm) and can thus be compensated by prism pairs [1–6], which are capable of generating significant anomalous GVD. For example, commercial devices such as the DeepSee (Newport) are capable of introducing  $-40,000 \text{ fs}^2$  of (anomalous) GVD at 690 nm and  $-8,000 \text{ fs}^2$  of GVD at 1,300 nm, which are generally sufficient for most microscopes at these wavelengths.

Three-photon microscopy (3PM) using 1,700 nm excitation wavelength was recently shown to produce images within *in vivo* brain tissue at unprecedented depths due to the higher order of nonlinearity and longer excitation wavelength than traditional 2PM [7]. The inverse-squared relationship between the generated signal and the duty cycle of the pulse train for 3PM, as opposed to the simple inverse relationship for 2PM [8,9], necessitates the use of short ( $<70 \text{ fs}$ ), energetic ( $\sim 100 \text{ nJ}$ ) excitation pulses. However, the GVD is anomalous for many glasses commonly used in microscopes at this wavelength, and a net anomalous GVD makes the use of typical glass prism pairs impractical for dispersion compensation. In addition, the immersion liquid (e.g.,  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$ ), and perhaps the brain tissue itself, can introduce anomalous dispersion and must also be considered.

Figure 1 shows the GVD of various optical materials as calculated from their Sellmeier equations [10–14]. The GVD curves of  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  only extend to 1.6  $\mu\text{m}$  because the Sellmeier coefficients in reference [14] were shown to match experiments to this wavelength.  $\text{H}_2\text{O}$  and  $\text{D}_2\text{O}$  as well as common types of glasses such as fused silica and N-LAK22 exhibit anomalous dispersion near 1,700 nm. However, the strong normal dispersion of silicon (Si) means that anomalous dispersion can be compensated by merely inserting a Si wafer into the beam path. Furthermore, the beam shouldn't suffer any transmission loss through the wafer if the wafer is undoped and uncoated as well as positioned at the Brewster angle, which is approximately at a 74 degree angle of incidence, and the incident light is p-polarized.

Second order autocorrelations were performed to measure how the laser pulse broadens after traveling through combinations of water ( $\text{H}_2\text{O}$ ), heavy water ( $\text{D}_2\text{O}$ ), Si, and the microscope. Figure 2 shows the experimental setup. The laser and PC rod combination produces a pulse of light centered at 1,700 nm with a 53 fs full-width at half maximum (FWHM). This initial pulse was measured by a second-order interferometric autocorrelator. After the interferometer, the light can pass through a variety of dispersive elements (1 mm cuvette filled with  $\text{H}_2\text{O}$ , 1 cm cuvette filled with  $\text{D}_2\text{O}$ , Si wafers of various thicknesses, and the microscope optics). Each sidewall of the cuvette is made of 1.25-mm thick fused silica, so each cuvette introduces approximately  $-100 \text{ fs}^2$  group delay dispersion (GDD). To achieve a 2 mm path length through  $\text{H}_2\text{O}$ , the incident light is double-passed through the 1-mm cuvette. A Si detector (SM05PD1A, Thorlabs) at the “Sample” position serves as the nonlinear element and produces a 2-photon induced current that is converted to voltage by a transimpedance amplifier (SR570, Stanford Research Systems) and then recorded by a computer.

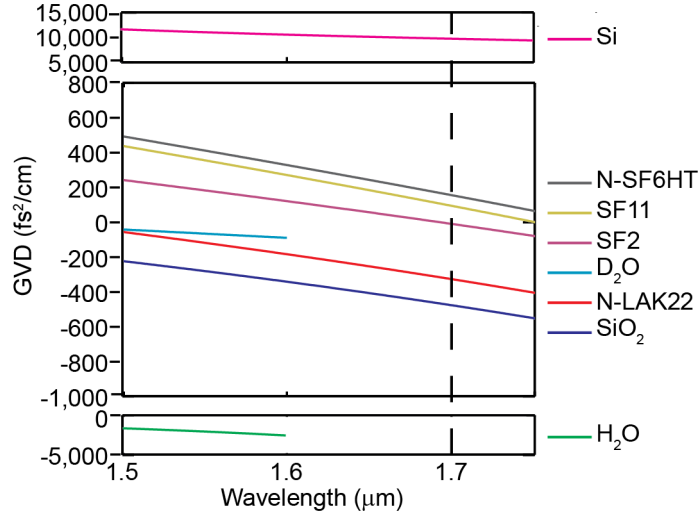


Fig. 1. Material GVD vs. wavelength near 1,700 nm for common optical materials. The vertical line at 1.7  $\mu\text{m}$  denotes our excitation wavelength. Note the different scales used for Si and  $\text{H}_2\text{O}$ .

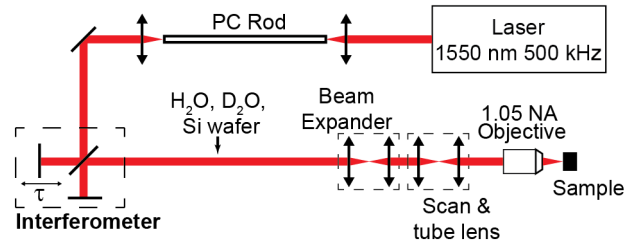


Fig. 2. Experimental setup. The PC Rod shifts the wavelength of the laser from 1.55  $\mu\text{m}$  to 1.7  $\mu\text{m}$  through soliton self-frequency shift [7]. Dispersive elements ( $\text{H}_2\text{O}$ ,  $\text{D}_2\text{O}$ , microscope optics, and Si wafers) were independently added to the beam path.

Figure 3 shows the pulse spectrum and 2nd-order interferometric autocorrelations of our experiment. The numbers in the upper-right corner of Fig. 3(b)-3(h) are the intensity FWHM of each pulse (after deconvolution by assuming a  $\text{sech}^2$  pulse shape). The dispersion introduced by 2 mm  $\text{H}_2\text{O}$  (Fig. 3(b)), which is the working distance of our objective, is nearly identical to that of 1 cm  $\text{D}_2\text{O}$  (Fig. 3(c)). We use  $\text{D}_2\text{O}$  as our 3PM immersion fluid because it has a similar linear index of refraction as  $\text{H}_2\text{O}$  but significantly lower absorption [14]. The small GVD that we observed from  $\text{D}_2\text{O}$ , which is corroborated by the calculated dispersion shown in Fig. 1, allows us to neglect its impact for 3PM since less than 2-mm thick  $\text{D}_2\text{O}$  is present in any imaging conditions. The thickness of the brain tissue in our experiments may also introduce extra dispersion. Since water content of brain tissue is approximately 80% [15], we performed our experiments with 1- and 2-mm  $\text{H}_2\text{O}$  together with our microscope. We found a 3-mm Si wafer is near the ideal thickness for our system (Figs. 3(f) and 3(h)). It is shown that the pulsewidth is compressed by over a factor of 2 after the 3-mm Si wafer, which should result in over a fourfold increase in the 3-photon excited signal.

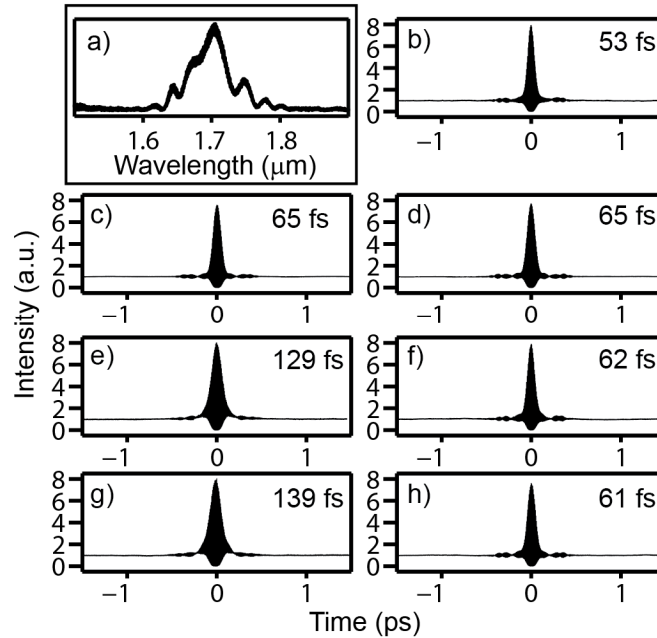


Fig. 3. Spectrum of pulse and second-order interferometric autocorrelations after various optical elements. (a) Pulse spectrum after the PC rod. (b)-(h) Second-order interferometric autocorrelations: (b) immediately after the collimating lens following the PC rod, (c) after 2 mm H<sub>2</sub>O, (d) after 1 cm D<sub>2</sub>O, (e) after microscope and 1 mm H<sub>2</sub>O, (f) after microscope, 1 mm H<sub>2</sub>O, and 3 mm Si, (g) after microscope and 2 mm H<sub>2</sub>O, (h) after microscope, 2 mm H<sub>2</sub>O, and 3 mm Si. The intensity FWHM of the pulse, assuming a  $\text{sech}^2$  pulse, is also displayed in each panel.

We performed *in vivo* imaging to show how the introduction of the Si wafer will increase the 3-photon excited signal for the same input beam. The microscope setup was similar to that shown in Horton et al. [7], with the exception of a PC rod with a larger core area that produces a soliton with roughly twice the pulse energy of the initial system [16].

We placed the 3 mm Si wafer (Edmund Optics) at the Brewster angle in order to minimize power loss. Transmitted power was measured after the microscope objective both before and after insertion of the wafer. No difference in the measured power was observed. The Brewster angle incidence increases the optical path length through the Si by approximately 4% of the wafer thickness.

Figure 4 shows the results of *in vivo* experiments recorded on the mouse brain. The mouse was prepared similar to that described in Horton et al. [7]. We used D<sub>2</sub>O as the immersion fluid because it has much lower absorption than that of H<sub>2</sub>O at 1,700 nm. The power at the surface was 1.4 mW when imaging 50 μm below the surface and 25 mW when imaging 900 μm below the surface. Deeper imaging was possible, but the depth at 900 μm was chosen because a large vessel provided a good measure for brightness comparison. Each depth shows approximately 4x increase in signal using the Si wafer, which agrees well with our pulse width measurements. It is important to note that the relative signal increase was similar at each depth despite different amounts of D<sub>2</sub>O and brain tissue involved. Our results indicate that fine-tuning the Si thickness at different imaging depth is unnecessary when imaging down to approximately 1 mm depth in a mouse brain using a 53 fs pulse at 1,700 nm.

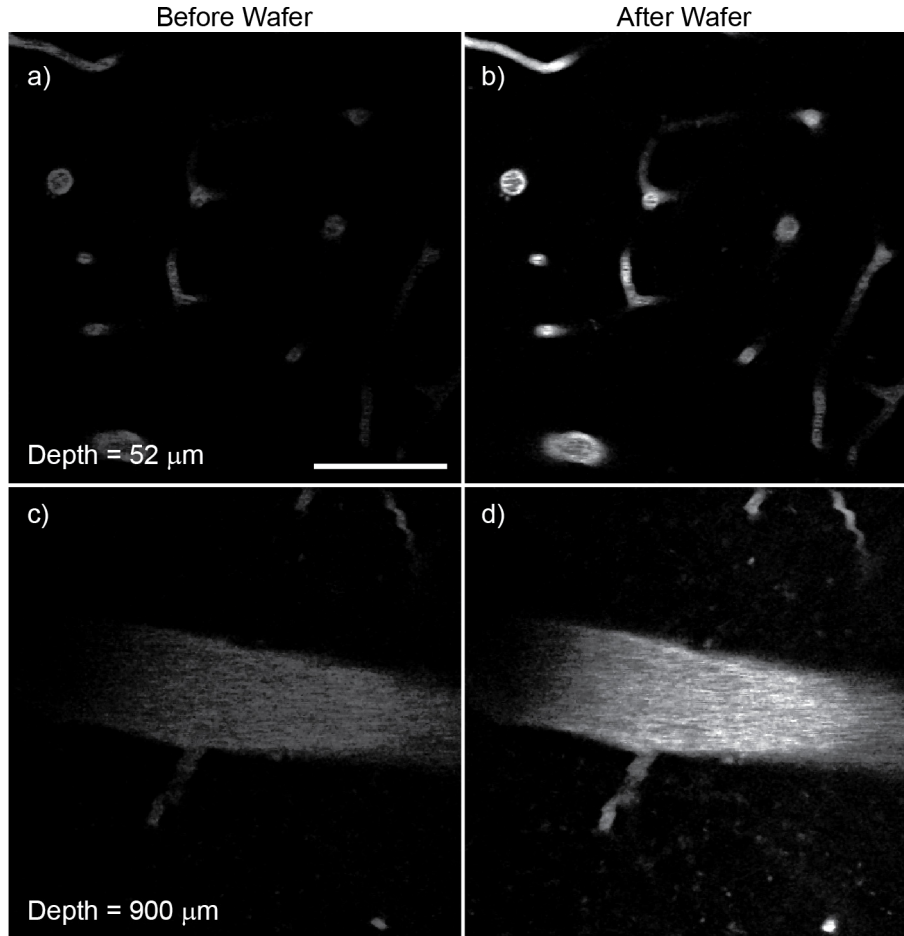


Fig. 4. In vivo three-photon microscopy of Texas Red-labeled blood vessel within an intact mouse brain. (a) and (c) were recorded without the Si wafer, while (b) and (d) were recorded after insertion of the 3 mm wafer at the Brewster angle. The brightness of the images reflects the signal level. Scale bar, 50  $\mu\text{m}$ .

## Discussion

We showed that the simple introduction of a Si wafer resulted in a 4x improvement of 3-photon excited signal for our three-photon microscope. Although fine-tuning the GDD is possible using Si prisms [6,17,18], adding a Si wafer to the optical beam path is significantly simpler. Since Si wafers of various thicknesses are readily available commercially, the addition of the Si wafer provides a simple, practical solution for dispersion compensation at 1,700 nm.

We were able to compress the pulse after the microscope to approximately 115% of the original pulse width using Si wafers at 0.5 mm thickness increments. The discrete nature of our dispersion compensation may introduce a maximum residual GDD of approximately 250  $\text{fs}^2$ , which can account for a fraction of the residual broadening (for example, we predict that 250  $\text{fs}^2$  will broaden the pulse by ~9% (~58 fs vs. ~53 fs). Pulse broadening due to third order dispersion (TOD) is a concern at 1,700 nm. TOD for common optical materials is displayed in Fig. 5. TOD is positive at 1,700 nm for many common glasses as well as Si, which means that Si cannot provide TOD compensation. Theoretical analysis shows that TOD of 30,000  $\text{fs}^3$  will cause a 15% increase in minimum pulsewidth for a 53 fs pulse. It is interesting to note

that many types of glasses have between 2000 to 2800 fs<sup>3</sup>/cm TOD (Fig. 5 and Table 1). Our imaging system includes approximately 12 cm of glass. Even though the exact types of glasses used in the microscope optics are not known, it is plausible that TOD could account for a fraction or all of the 15% minimum pulse width increase.

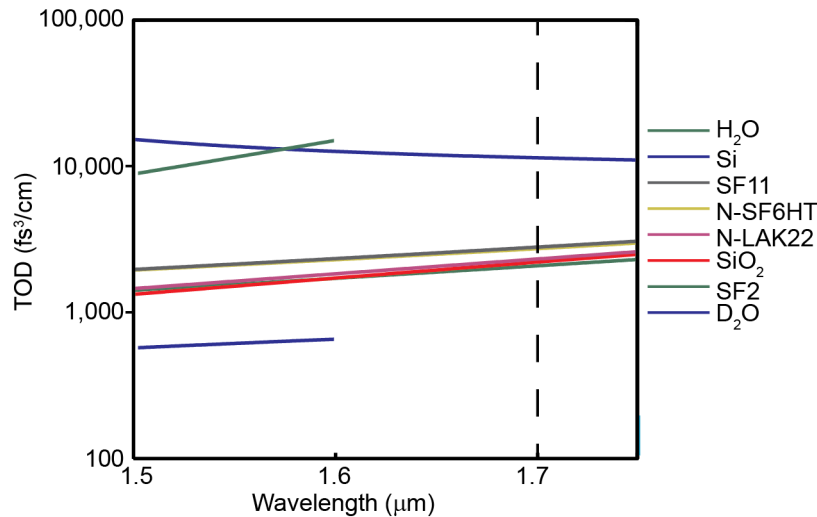


Fig. 5. TOD of common optical materials.

**Table 1. Calculated GVD and TOD of various materials at 1,700 nm. The bold values are calculations at 1,600 nm**

Material	GVD (fs <sup>2</sup> /cm)	TOD (fs <sup>3</sup> /cm)
<b>H<sub>2</sub>O</b>	<b>-2,525</b>	<b>14,932</b>
Si	9,827	11,406
SF11	95	2,795
N-SF6HT	156	2,727
N-LAK22	-326	2,317
NBK7	-448	2,261
Fused Silica	-475	2,206
SF2	-9	2,082
<b>D<sub>2</sub>O</b>	<b>-89</b>	<b>660</b>

In summary, we show that a Si wafer of appropriately chosen thickness can conveniently compensate for the anomalous dispersion introduced by the optical components in a three-photon microscope at 1,700 nm. A factor of over two pulsewidth reduction at the sample is obtained by the simple addition of a 3-mm Si wafer at Brewster angle incidence, which leads to a factor of 4 improvement in signal for three-photon *in vivo* imaging. Our results further indicate that fine-tuning the Si thickness is unnecessary as a function of imaging depth when imaging down to approximately 1 mm depth in a mouse brain using a 53 fs pulse at 1,700 nm. Finally, TOD generated by glass optics and H<sub>2</sub>O may be a concern at 1,700 nm, particularly if a pulse width much shorter than 50 fs is used.

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